IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Jan Wadstein *et al.*
Serial No.: 09/410,484 Group No.: 1614
Filed: 09/30/99 Examiner: Webman
Entitled: **Method Of Treating Hypertension And Reducing Serum Lipase Activity**

Declaration of Asgeir Sæbo

Assistant Commissioner for Patents
Washington, D.C. 20231

CERTIFICATE OF MAILING UNDER 37 C.F.R. § 1.8(a)(1)(i)(A)

I hereby certify that this correspondence (along with any referred to as being attached or enclosed) is, on the date shown below, being deposited with the U.S. Postal Service with sufficient postage as first class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231.

Dated: 4-25-07 By: _____

I, Asgeir Sæbo, state as follows:

1. My present position is Research Director, Natural ASA.

2. It is my understanding that the Examiner has stated the following in the Office Action dated January 25, 2007:

> First, Kawamura et al. provide a nexus teaching between weight loss in hypertensive patients and lowering of blood pressure. It remains obvious to one of ordinary skill in the art that the method of Cook et al. can lower blood pressure via weight loss. The Examiner did not assert that CLA would have a positive or negative on hypertension like ephedrine as submitted by Applicant. The Examiner asserts that weight loss is directly related to lowering of blood pressure, as supported by Kawamura et al., and CLA can be used to effect weight loss as taught by Cook et al. Secondly, one of ordinary skill in the art would have a reasonable expectation of success because of the nexus teaching of Kawamura et al.

3. When a biologically active agent, such as CLA, is administered to a subject there can be a variety of effects. Just because CLA causes weight loss does not also mean that it would reduce hypertension. A person of skill in the art would not reasonable expect CLA to reduce hypertension for two reasons.

4. First, CLA has been shown to elevate the level of F2-isoprostane. Taylor et al., Conjugated Linoleic Acid Impairs Endothelial Function, Arteriosclerosis, Thrombosis, and Vascular Biology 26(2), 307-312 (2006)(attached at Tab 1). F2-isoprostanes have a vasoconstrictive effect. Cracowski et al., Cardiovascular pharmacology and physiology of the isoprostanes, Fundamental & Clinical Pharmacology 20(5): 417-427 (2006)(attached at Tab 2). Taken together, it should be expected that administration of CLA would result in an increase in blood pressure.

5. Second, administration of other agents known to be effective for weight loss can result in increased hypertension. Ephedrine, a commonly used, biologically active weight loss supplement is one such example. As established in Haller and Benowitz, Adverse Cardiovascular and Central Nervous System Events Associated with Dietary Supplements Containing Ephedra Alkaloids, New England J. Med. 343(25):1833-1838 (2000)(attached at Tab 3), ephedrine can cause an increase in hypertension.

6. The Examiner's argument that it would be obvious to use CLA to decrease hypertension because CLA administration also causes weight loss lacks scientific merit. How an agent such as CLA acts in the body is complex. Whether CLA causes an increase or decrease in hypertension, or has no effect at all, is determined by a variety of factors that have no relation to weight loss. It is not scientifically valid to draw a conclusion that because an agent causes weight loss, it can also be expected to decrease hypertension. The references cited by the Examiner contain no data that can be interpreted in this manner.

7. I further declare that all statement made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of title 18 of the United States

Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Asgeir Sæbo

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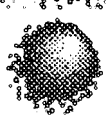

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REVIEW ARTICLE

Cardiovascular pharmacology and physiology of the isoprostanes

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ABSTRACT

F_2-isoprostanes are a complex family of compounds produced from arachidonic acid via a free radical-catalyzed mechanism. Their quantification as a pathophysiological biomarker provides a unique opportunity to investigate lipid peroxidation in vascular diseases. Their measurement also provides an interesting biomarker for the rational dose selection of antioxidants in vascular diseases where oxidative stress might be involved. In addition to their use as biomarkers, some isoprostanes possess a biological activity. The 15-series F_2- and E_2-isoprostanes mediate vasoconstriction in different vascular beds and species. In addition, 15-F_{2t}-IsoP induces smooth muscle cells mitogenesis and monocyte adhesion to endothelial cells. The data available supports but does not prove the hypothesis that isoprostanes are involved in vascular physiology and pathogenesis.

INTRODUCTION

Isoprostanes are a complex family of compounds produced from arachidonic acid via a free radical-catalyzed mechanism. In vitro generation of auto-oxidation products derived from polyunsaturated fatty acids was described more than 30 years ago [1,2]. However, the first demonstration that these compounds were produced in humans was shown in 1990 by Morrow et al. [3], who reported the discovery of prostaglandin-F_2-like compounds, termed F_2-isoprostanes, generated by free radical-induced peroxidation of arachidonic acid. Since that time, F_2-isoprostanes have been used extensively as clinical markers of lipid peroxidation in cardiovascular disorders. These compounds are not only biomarkers. Indeed, the 15-series F_2- and E_2-isoprostanes possess a pharmacological activity on the blood vessels.

ISOPROSTANE STRUCTURE AND SYNTHESIS

Depending on which of the labile hydrogen atoms of arachidonic acid is first abstracted by free radicals, three initial arachidonoyl radicals can be formed following free radical attack. These radicals form four prostaglandin-H_2-like compounds that can then be fully reduced to form four prostaglandin $F_{2\alpha}$ regioisomers (*Figure 1*), or rearranged to form prostaglandin E_2 and D_2 regioisomers. Two mechanisms, based on the formation of a 'dioxetane' intermediate, via a 4-*exo*-cyclization or a β-fragmentation followed by successive 5-*exo*-cyclizations have been proposed recently for the formation of these compounds [4]. Because each F_2-isoprostane regioisomer comprises eight diastereoisomers, 64 different F_2-isoprostanes can be generated.

Isoprostanes were formerly named according to the prostaglandin $F_{2\alpha}$ chemical structure. They differ from prostaglandins by the *cis*-stereochemistry of the five-membered ring junction instead of the *trans*-stereochemistry of the prostaglandin $F_{2\alpha}$. Because the first isoprostanes described were the 15-series, they were formerly named according to this major difference, the first isoprostane being named 8-*iso*-prostaglandin $F_{2\alpha}$ or 8-*epi*-prostaglandin $F_{2\alpha}$. However, such a nomenclature does not allow the differentiation of the numerous isomeric structures. Two nomenclatures were proposed

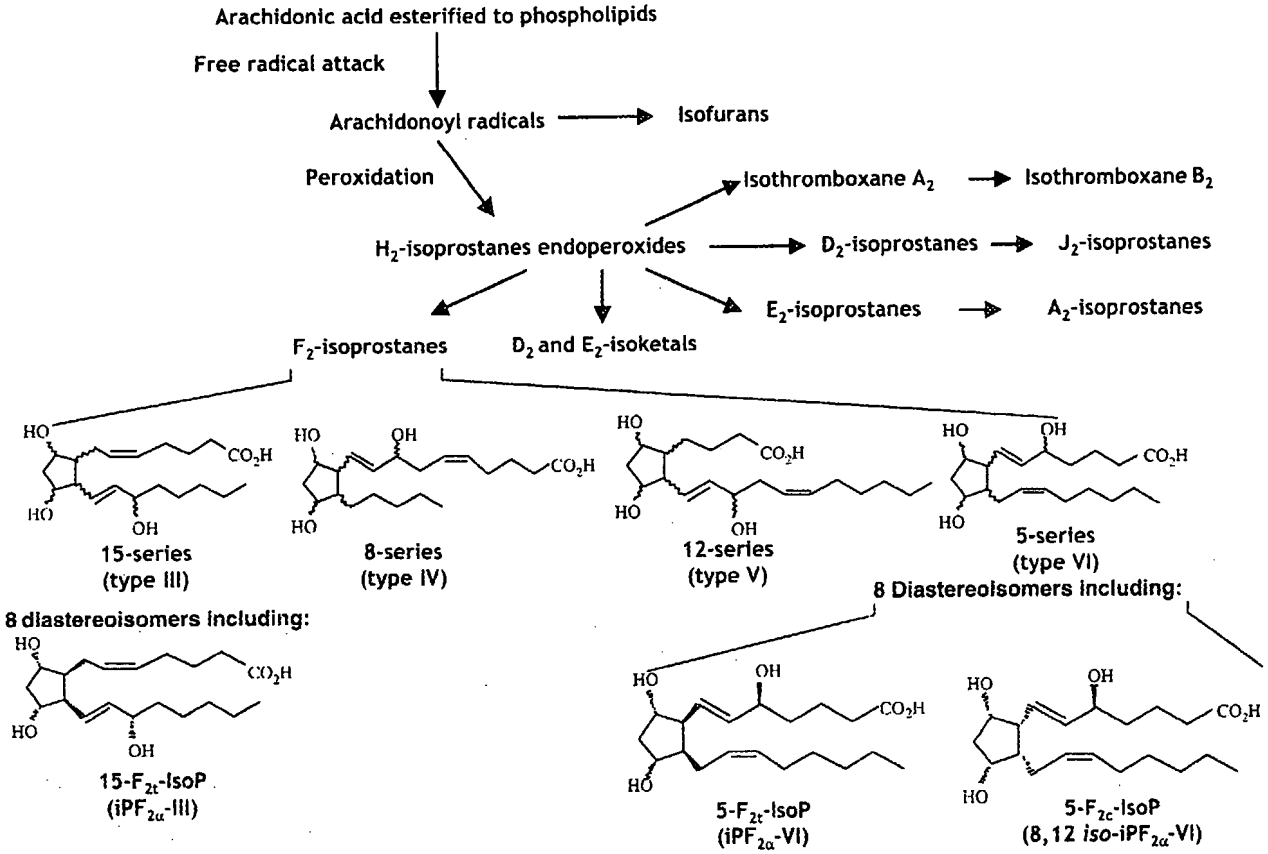


Figure 1 The isoprostane (IsoP) pathway. Free radical attack of arachidonic acid results in the formation of arachidonoyl radicals, which, following peroxidation, form four prostaglandin-H_2-like compounds that can then be fully reduced to form four prostaglandin $F_{2\alpha}$ regioisomers [those of the 15-series (type III), 8-series (type IV), 12-series (type V) and 5-series (type VI)], or rearranged to form prostaglandin E_2 and D_2 regioisomers. Each regioisomer comprises eight diastereoisomers and so 64 different F_2-isoprostanes can be generated.

recently, both of which enable an easy differentiation of the isoprostane isomers. Taber et al. [5] nomenclature was filed with the Eicosanoid Nomenclature Committee, and approved by the International Union of Pure and Applied Chemistry. Rokach et al. [6] also proposed a nomenclature that enables the differentiation of the regioisomers. In 1997, this nomenclature was modified to be applicable to isoprostane-like compounds derived from eicosapentaenoic and docosahexaenoic acid. The concomitant use of these three different nomenclatures is confusing for the nonspecialist, and we propose that the old nomenclature (e.g. 8-*iso*-prostaglandin $F_{2\alpha}$) be definitively abandoned, and that Taber's nomenclature be encouraged. The different nomenclatures used to name the main isoprostanes are given in *Figure 2*.

Several in vitro studies have suggested a cyclooxygenase (COX)-dependent formation of 15-F_{2t}-IsoP [7–9]. An efficient in vivo production of 15-F_{2t}-IsoP through the COX pathway would reduce its accuracy as a valid marker of lipid peroxidation. In contrast to the in vitro data, clinical studies clearly showed that COX inhibition was unable to decrease the formation of F_2-isoprostanes in healthy subjects as well as patients, suggesting that F_2-isoprostanes are formed via a non-COX-dependent mechanism in vivo [10–14]. Furthermore, in conditions of increased COX-2 expression following intravenous lipopolysaccharide challenge, the formation of 15-F_{2t}-IsoP and of 5-series isomers was not altered by COX inhibitors in healthy volunteers, whereas prostanoid production was decreased, further suggesting a COX-independent pathway of F_2-isoprostane synthesis [15]. Finally, an in vivo COX-dependent formation of iPF2-III has been shown in the rat but not in humans [16]. Altogether, these data suggest that although a COX-dependent formation can be demonstrated in vitro, this does not occur in vivo in humans, meaning that 15-F_{2t}-IsoP as well as the 5-series isomers can be used as biomarkers of lipid peroxidation in vivo.

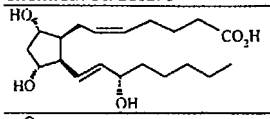
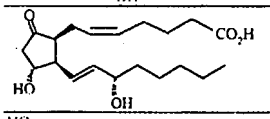
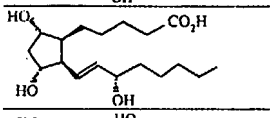
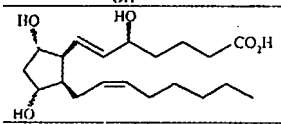
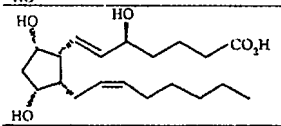
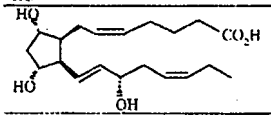
Chemical structure	Former nomenclature	Taber's nomenclature	Rokach's nomenclature
	8-*iso*-$PGF_{2\alpha}$	15-F_{2t}-IsoP	$iPF_{2\alpha}$-III
	8-*iso*-PGE_2	15-E_{2t}-IsoP	iPE_2-III
	2,3-dinor-5,6-dihydro-8-*iso*-$PGF_{2\alpha}$	2,3-dinor-5,6-dihydro-15-F_{2t}-IsoP	2,3-dinor-5,6-dihydro-$iPF_{2\alpha}$-III
	Not available	5-F_{2t}-IsoP	$iPF_{2\alpha}$-VI
	Not available	5-F_{2t}-IsoP	8,12-*iso*-$iPF_{2\alpha}$-VI
	8-*iso*-$PGF_{3\alpha}$	15-F_{3t}-IsoP	$iPF_{3\alpha}$-III

Figure 2 Nomenclature of the isoprostanes.

ISOPROSTANE QUANTIFICATION

Quantification of F_2-isoprostanes is used as a reliable marker of lipid peroxidation in vivo [17], and several methods are currently used [18] including gas chromatography (GC)–mass spectrometry (MS), which might be associated with an immunoaffinity extraction, GC–tandem MS, and liquid chromatography–tandem MS. These methods are reviewed in detail elsewhere [19]. They are specific but their cost and technology limit their routine use. Measurement of urinary 15-F_{2t}-IsoP by radioimmunoassay has been validated and constitutes a valid and easier alternative to GC–MS [20]. Enzyme immunoassays have also been developed to measure levels of F_2-isoprostanes but the antibodies used have not been tested for cross-reactivity with the numerous F_2-isoprostane isomers and their metabolites. The results obtained using enzyme immunoassays sometimes differ from those obtained using GC–MS assays and therefore immunoassays should be considered as semi-quantitative indices of F_2-isoprostanes.

VASCULAR PHARMACOLOGY OF THE ISOPROSTANES

Isoprostanes are formed in situ on phospholipids, at sites of free radical generation. Once released from cell membranes by phospholipases, isoprostanes circulate in the plasma in free forms and are therefore susceptible to activate membrane receptors. Most studies have focused on the biological activity of 15-F_{2t}-IsoP, the first isoprostane commercially available. 15-F_{2t}-IsoP is a vasoconstrictor in most species and vascular beds, both in vitro and in vivo, following intravenous administration (see [21,22] for full reviews). These constrictor properties are not specific to the blood vessels and have been demonstrated in the lymphatic vessels, the bronchi, the gastrointestinal tract and the uterus. In addition, it stimulates mitogenesis in uterine vascular smooth muscle cells [23]. The available data strongly suggest that the effects of 15-F_{2t}-IsoP on blood vessels are mediated by the activation of the TP receptors (thromboxane A_2/prostaglandin H_2 receptors), acting as a full or partial agonist [24,25], although some responses including mitogenesis appear to be at least in part TP receptor independent. The existence of a specific isoprostane receptor has been suggested but remains to be elucidated [26]. In addition, preliminary data suggest that isoprostanes, as well as their precursor arachidonic acid, are other lipid ligands for the peroxisome proliferator activated receptors (PPAR) [27].

The effects of 15-F_{2t}-IsoP on platelets are complex. When incubated with subthreshold concentrations of ADP, thrombin, collagen and arachidonic acid, 15-F_{2t}-IsoP causes irreversible platelet aggregation, dependent

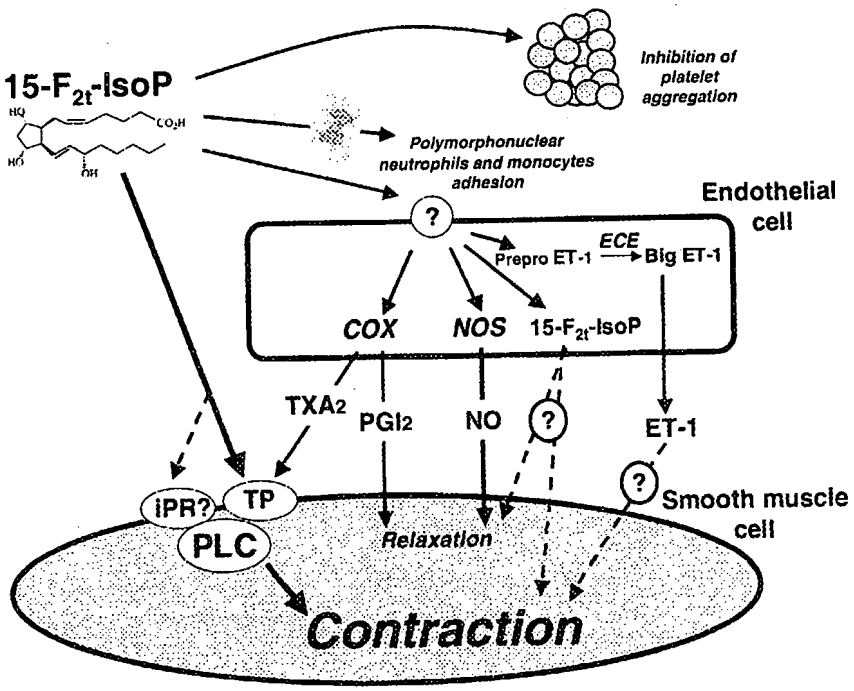


Figure 3 Schematic representation of the pharmacological activities of the isoprostane 15-F_{2t}-IsoP on the interface blood vessels. COX, cyclo-oxygenase; TXA_2, thromboxane A_2; PGI_2, prostacyclin; NO, nitric oxide; TP, prostaglandin H_2/thromboxane receptor; iPR, specific isoprostane receptor; PLC, phospholipase C; ET-1, endothelin-1; ECE, endothelin conversion enzyme. For a better comprehension, the thickness of the arrows are correlated to the scientific evidence of these mechanisms. Such mechanisms are likely to differ within species, as well as within the vessel types.

on thromboxane generation, while 15-F_{2t}-IsoP alone induces weak, reversible aggregation, only at high concentrations [28]. As 15-F_{2t}-IsoP is a partial agonist at the prostanoid TP receptor on platelets, it might inhibit the pro-aggregatory effects of TP receptor stimulation. Indeed, in human whole blood, 15-F_{2t}-IsoP is anti-aggregatory [29]. Several authors suggested that increased isoprostane formation is one of the factors involved in aspirin resistance, but a full demonstration of this hypothesis is not available to date [30–33].

15-F_{2t}-IsoP-induced contraction is modulated by the endothelium through the release of NO, i.e. endothelium removal increases 15-F_{2t}-IsoP contraction [21]. In addition, 15-F_{2t}-IsoP induces both thromboxane A_2 and endothelin-1 release from endothelial cells (*Figure 3*). In comparison with the huge data available for 15-F_{2t}-IsoP, few are available for other isomers. Nevertheless, other isoprostanes belonging to the 15 series of the F-family isoprostanes, such as 9-epi-15-F_{2t}-IsoP and 15-epi-15-F_{2t}-IsoP are biologically active, although less potent than 15-F_{2t}-IsoP [21]. The 5-series and 15-series F_2-isoprostanes are produced in approximately equal amounts in vivo whereas the 8-series and 12-series F_2-isoprostanes are produced in lower amounts [34]. In human urine and plasma, the 5-series (e.g. 5-F_{2t}-IsoP and 5-F_{2c}-IsoP) was found to be the most abundant F_2-isoprostanes [35]. Both the 15-series and the 5-series are easily detectable in human urine and plasma. However, in contrast to the 15-series F_2-isoprostanes, the 5-series F_2-isoprostanes have no vasomotor effect in different species and vascular beds [36] and as such are unlikely to be involved in the pathogenesis of vascular diseases. In addition to the vasoconstrictor and mitogenic effects, 15-F_{2t}-IsoP and 15-E_{2t}-IsoP induce monocyte adhesion to endothelial cells [37,38], whereas 15-F_{2t}-IsoP is a specific activator of rapid neutrophil adhesion [39]. Furthermore, 15-F_{2t}-IsoP induces cerebral endothelial cell death [40]. Endothelial cells are one of the first targets of oxidative stress in atherogenesis and ischemia–reperfusion injury. Whether isoprostanes may be one of pathogenic mediators remains to be tested.

Compared with the F_2-isoprostanes, E_2-isoprostanes are more potent in vitro. 15-E_{2t}-IsoP is more potent than 15-F_{2t}-IsoP in systemic and pulmonary vessels, its contraction being mediated through TP receptor, and EP_3 receptor activation in the pulmonary vasculature [41,42]. In addition, 15-E_{2t}-IsoP may induce a relaxation through EP receptors [43]. However, because no data are available concerning the production of E_2-isoprostanes in cardiovascular disorders, it is premature to conclude concerning the potential role of such compounds in cardiovascular pathogenesis.

One should keep in mind that Morrow et al. showed [44] that A_2-isothromboxanes are formed in vivo. Due to the inherent instability of the thromboxane A_2 ring, no data concerning the vascular effects of A_2-isothromb-

oxanes are available. However, regarding the potency of thromboxane A_2-induced contractions, studies using stable A_2-isothromboxanes analogues are awaited with interest.

To date, the metabolism of 15-F_{2t}-IsoP leads to two major metabolites in humans: 2,3-dinor-15-F_{2t}-IsoP and 2,3-dinor-5,6-dihydro-15-F_{2t}-IsoP [45,46]. The metabolite 2,3,4,5-tetranor-13,14-dihydro-15-keto-15-F_{2t}-IsoP [47] was identified as the major metabolite in rabbits but there is no evidence that such a metabolite is produced in humans. Although most prostanoid metabolites are biologically inactive, surprisingly, a recent report showed that 2,3-dinor-5,6-dihydro-15-F_{2t}-IsoP exhibits contraction that is comparable with that of 15-F_{2t}-IsoP in porcine brain microvessels [48]. By contrast, unlike 15-F_{2t}-IsoP, neither 2,3-dinor-5,6-dihydro-15-F_{2t}-IsoP nor 2,3-dinor-15-F_{2t}-IsoP had any constrictor or dilator effects on the rat thoracic aorta [49]. Such disparate observations need to be further investigated in different species and vascular beds.

Recently, isoprostane-like compounds derived from eicosapentaenoic and docosahexaenoic acids have been discovered in vivo [50–52]. Among the F_3-isoprostanes formed, 15-F_{3t}-IsoP possesses either no biological effect or might induce a weak relaxation in human airways. Preliminary data suggest that F_4-neuroprostanes possess no vascular effects.

ISOPROSTANES AS A BIOMARKER OF LIPID PEROXIDATION IN VASCULAR DISEASES

Isoprostanes have been measured in biological fluids such as urine, plasma, exhaled breath condensate, bronchoalveolar lavage fluid, bile, cerebrospinal, seminal and pericardial fluids. They are also detectable in normal tissues, including umbilical cords [53]. The main advantage of urinary measurements is that both 15-F_{2t}-IsoP and 5-F_{2t}-IsoP are not formed ex vivo by auto-oxidation in urine, unlike in plasma samples.

Cigarette smoking was one of the first conditions in which an increase in F_2-isoprostane levels was demonstrated [54]. This increase is reduced after 2 weeks of abstinence from smoking [14,54] and almost reaches the values of nonsmokers 4 weeks after quitting smoking [55]. Short-term cigarette smoking increases exhaled breath condensate F_2-isoprostane concentrations [56], but not plasma levels of F_2-isoprostane [54]. Restarting smoking after quitting and passive smoking are associated with an increase in plasma levels of F_2-isoprostane [57,58]. Interestingly, 15-F_{2t}-IsoP concentrations were approximately twice as high in umbilical cords from newborn babies of smoking mothers compared with those of nonsmoking mothers [53]. Together, these data provide evidence that cigarette smoking is associated with a chronic increased lipid peroxidation in vivo.

The measurement of isoprostanes in biological fluids has prompted clinical investigations on the pathophysiological role of lipid peroxidation in cardiovascular diseases (*Table 1*). Among the biological fluids available, most studies were performed on urine because of the non-invasiveness of the procedure and the lack of artifactual generation. A strong link between lipid peroxidation and vascular diseases associated with ischemia–reperfusion, atherosclerosis and inflammation has been suggested by the elevated levels of lipid peroxidation observed in such diseases.

In addition to being a pathophysiological marker, the quantification of F_2-isoprostanes might represent a prognostic marker. Indeed, Schwedhelm et al. [59] showed in a case–control study that urinary 15-F_{2t}-IsoP level was a strong independent concentration-dependent risk marker of coronary heart disease. In addition, there is a relationship between plasma F_2-isoprostanes and early development of coronary artery calcifications [60]. There are currently no published clinical studies aimed at testing isoprostanes as a long-term prognostic marker, with strong endpoints such as mortality or morbidity, but cohort studies are on-going.

ISOPROSTANES: EMERGING ROLE IN VASCULAR PHYSIOLOGY AND DISEASE?

An important issue to resolve is whether the same effects observed in vitro are observed consistently in vivo at physiological concentrations and whether these effects contribute to pathological states in vivo. Basal plasma concentrations of 15-F_{2t}-IsoP have been found to range from approximately 10^{-10} to 5×10^{-10} mol L^{-1} in plasma samples. These concentrations are unlikely to induce a systemic vasoactive effect considering the EC_{50} values of 15-F_{2t}-IsoP observed in vitro [21,61]. However, F_2-isoprostanes are released at the site of free radical injury and then diluted in the circulation and therefore local concentrations might be sufficiently high to induce regional vasoconstriction. The concentrations of 15-F_{2t}-IsoP and 5-F_{2t}-IsoP are increased markedly in the coronary sinus following coronary angioplasty [62]. However, 15-F_{2t}-IsoP concentrations are in the

Table 1 F_2-isoprostane quantification in human cardiovascular diseases.

Disease	Isoprostanes quantified	Tissue or biological fluid tested	Method	Results in comparison with a control group	References
Essential hypertension	15-F_{2t}-IsoP	Urine	EIA and GC–MS	NSD	[86–88]
Hypertensive patients with renovascular disease	15-F_{2t}-IsoP	Urine	EIA	Increased	[86]
Heart failure	15-F_{2t}-IsoP	Urine, pericardial fluid	LC–MS–MS and EIA	Increased	[89–92]
Atherosclerosis	15-F_{2t}-IsoP and 5-F_{2t}-IsoP	Atherosclerotic lesions from carotid endarterectomy	GC–MS and RIA	Increased	[93–95]
Ruptured abdominal aortic aneurysm	15-F_{2t}-IsoP	Plasma	EIA	Increased	[96]
Stable coronary heart disease	15-F_{2t}-IsoP	Urine	GC–MS and RIA	NSD	[97,98]
Unstable angina	15-F_{2t}-IsoP	Urine	RIA	Increased	[98]
Reperfusion following myocardial infarction and cardiopulmonary bypass	15-F_{2t}-IsoP and 5-F_{2t}-IsoP	Urine	GC–MS	Increased	[97,99]
Coronary angioplasty	15-F_{2t}-IsoP and 5-F_{2t}-IsoP	Urine and coronary sinus	GC–MS	Increased	[62,99]
Systemic sclerosis (scleroderma)	15-F_{2t}-IsoP and F_2-isoprostane metabolites	Urine	GC–MS AND EIA	Increased	[100–104]
Antiphospholipid antibodies syndrome	15-F_{2t}-IsoP and 5-F_{2t}-IsoP	Urine	GC–MS and EIA	Increased	[105,106]
Raynaud's phenomenom	15-F_{2t}-IsoP	Urine	GC–MS	NSD	[101,104]
Pulmonary hypertension	15-F_{2t}-IsoP	Urine	GC–MS	Increased	[107]
Acute ischemic stroke	15-F_{2t}-IsoP	Urine	RIA	No variation over 72 h	[108]
Migraine	15-F_{2t}-IsoP	Urine	RIA	NSD	[109]
Preeclampsia	15-F_{2t}-IsoP and 5-F_{2c}-IsoP	Plasma, urine and saliva and placental tissue	GC-MS and EIA	Conflicting results among studies	[110–116]

EIA, enzyme immunoassay; GC–MS, gas chromatography–mass spectrometry; LC–MS–MS, liquid chromatography–tandem mass spectrometry; NSD, not significantly different; RIA, radioimmunoassay.

nanomolar range, and thus unlikely to contribute to epicardial coronary artery vasoconstriction [61,63].

No specific inhibition of 15-F_{2t}-IsoP or other isoprostanes vascular effects can currently be achieved. However, TP receptor antagonists but not aspirin are effective in atherosclerosis inhibition in apo E knock-out mice, showing that TP receptors blockade by S18886 is effective by a mechanism independent of platelet-derived thromboxane A_2 [64], whereas isoprostanes suppression with vitamin E retards atherogenesis in the same animal model [65]. Similarly, TP receptor antagonism by L670596, but not COX-2 inhibition prevented pulmonary hypertension and endothelin-1 upregulation in 60% O_2-mediated pulmonary hypertension in newborns rats [66]. In addition to these animal data, a recent study showed that in patients suffering from coronary artery disease, S18886, a TP receptor antagonist improved acetylcholine-induced and flow-mediated vasodilation in patients treated with aspirin [67]. An hypothesis is that endogenous TP receptor activation induced by 15-F_{2t}-IsoP or other isoprostanes may be involved in the COX-independent effects of TP receptors antagonists [68]. However, because TP receptors share other endogenous ligands such as prostaglandin H_2 or hydroxyeicosatetraenoic acids (HETEs), such data give strength to the hypothesis that isoprostanes are involved in the vascular physiology and pathogenesis, but does not enable a definitive conclusion. Given that many isomeric isoprostanes exist, one should not focus only on 15-F_{2t}-IsoP. Other 15-series F_2-isoprostanes are biologically active, as well as E_2-isoprostanes [22] and large field of investigations are still unexplored.

ISOPROSTANES AS A PHARMACOLOGICAL TOOL FOR DRUG EVALUATION

In the past decade, most attention has focused on the effect of an antioxidant therapeutic strategy, including the use of vitamin E, in cardiovascular and nephrological diseases, with mixed results. Measurement of F_2-isoprostanes currently represents a valuable pharmacological tool for the evaluation of antioxidant therapy, and should be used in the rational selection of antioxidant dosages. Vitamin E supplementation induced a reduction of urinary 15-F_{2t}-IsoP levels in patients with cystic fibrosis [69], type 2 diabetes [11] and homozygous homocystinuria [70], but not in patients with systemic sclerosis [71]. In addition, such a reduction was dose dependent in hypercholesterolemic patients [10]. The vitamin E-dependent reduction in the concentrations of F_2-isoprostane was observed in clinical conditions of enhanced oxidative stress. By contrast, supplementation with vitamin E had no effects on F_2-isoprostane levels in either moderate cigarette smokers [72], or healthy adults [73]. In the latter studies, the supplementation was associated with a significant dose-dependent increase in circulating concentrations of vitamin E. In addition, vitamin E supplementation in cigarette smokers on a high polyunsaturated fatty acid diet caused an increase in the plasma levels of F_2-isoprostane [74]. Together, these studies suggest that vitamin E supplementation has antioxidant effects in patient populations that are characterized by high rates of lipid peroxidation. Patrignani et al. [72] hypothesized that the basal rate of lipid peroxidation may be an important determinant of the response to vitamin E supplementation, and could explain the variable effects of vitamin E supplementation in large clinical trials. Several studies favor such a hypothesis. Oral treatment with raxofelast, a vitamin-E-like antioxidant, induced a significant reduction of plasma concentrations of 15-F_{2t}-IsoP in type 2 diabetes but had no effect in healthy subjects [75]. In addition, vitamin C supplementation reduced urinary 15-F_{2t}-IsoP levels in patients with stroke [76], and chronic alcoholic liver disease but not in patients with hepatitis C cirrhosis, in whom endogenous vitamin C and E concentrations did not significantly differ compared with controls [77]. Furthermore, vitamin C did not decrease urinary F_2-isoprostane and metabolite levels in healthy young women [78], and in young subjects with a limited history of cigarette smoking [79]. Together, these data strengthen the need to incorporate the measurement of surrogate end-points such as F_2-isoprostanes in large-scale antioxidant clinical trials.

In addition to drug evaluation, F_2-isoprostane measurement could be used to test the antioxidant properties of the diet. F_2-isoprostane levels were decreased following supplementation with eicosapentaenoic acid or docosahexaenoic acid [80], fish meals in diabetic patients [81], olive oil [82], soy-containing isoflavone [83], gazpacho [84], and flavanol-rich cocoa [85].

CONCLUSION

Isoprostanes are a complex family of compounds produced from arachidonic acid via a free radical-catalyzed mechanism. Some isoprostanes induce vasoconstriction, mitogenesis and monocyte adhesion. The quantification of F_2-isoprostanes as a pathophysiological biomarker

provides a unique opportunity to investigate lipid peroxidation in vascular diseases. Their measurement provides an interesting biomarker for the rational dose selection of antioxidants in vascular diseases where oxidative stress might be involved. The data available supports but does not prove the hypothesis that isoprostanes are involved in vascular physiology and pathogenesis.

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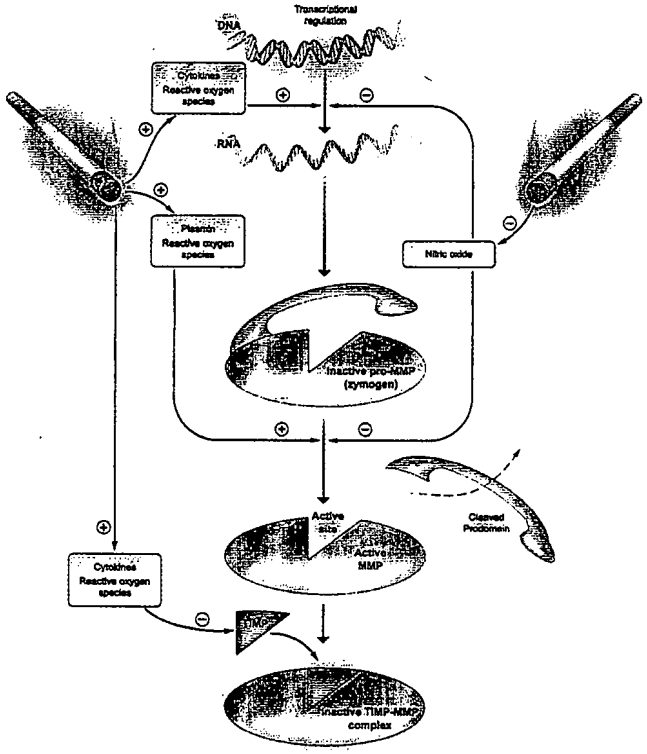

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Brief Reviews

Vascular Biology

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Conjugated Linoleic Acid Impairs Endothelial Function

Justin S.W. Taylor, Simon R.P. Williams, Rhian Rhys, Phillip James, Michael P. Frenneaux

Objectives—To determine the effect of dietary supplementation with conjugated linoleic acid (CLA) on body mass index (BMI), body fat distribution, endothelial function, and markers of cardiovascular risk.

Methods and Results—Forty healthy volunteers with BMI >27 kg/m^2 were randomized to receive a CLA isomeric mixture or olive oil in a 12-week double-blind study. Subcutaneous body fat and abdominal/hepatic fat content were assessed using skin-fold thicknesses and computed tomography scanning, respectively. Endothelial function was assessed by brachial artery flow-mediated dilatation (FMD). Plasma isoprostanes were measured as an index of oxidative stress. CLA supplementation did not result in a significant change in BMI index or total body fat. There was a significant decrease in limb (−7.8 mm, $P<0.001$), but not torso skin-fold thicknesses or abdominal or liver fat content. Brachial artery FMD declined (−1.3%, $P=0.013$), and plasma F2-isoprostanes increased (+91pg/mL, $P=0.042$).

Conclusions—A CLA isomeric mixture had at most modest effects on adiposity and worsened endothelial function. On the basis of these results, the use of the isomeric mixture of CLA as an aid to weight loss cannot be recommended. (*Arterioscler Thromb Vasc Biol.* 2006;26:307-312.)

Key Words: body composition ■ conjugated linoleic acid ■ endothelial function ■ obesity ■ oxidative stress

Abdominal obesity[1] and the associated dysmetabolic syndrome[2] confer increased cardiovascular risk. Dietary modification with n-3 polyunsaturated fatty acids appears to reduce the risk of coronary artery disease and improve mortality.[3-5] Conjugated linoleic acid (CLA) is a naturally occurring fatty acid and is found in dairy products and meat from ruminants. It differs from the better known linoleic acid by having an extra carbon-carbon double bond. It has been widely promoted in the lay press,[6] with claims that it can prevent and treat cancer,[7] prevent heart disease,[8] improve immune function,[9] and treat obesity.[10] Whereas some of these effects are supported by studies in animals,[8,11] there is conflicting published human research on CLA, and in particular there have been no conclusive studies measuring its effect on markers of cardiovascular risk. Furthermore, it is now realized that the different isomers of CLA may have very different biological properties and may have different mechanisms of action.[12] The 2 main CLA isomers that have been studied are 9,11 and 10,12 CLA. Other studies have suggested that CLA supplementation may increase oxidative stress, although this was not proven.[16,17] Furthermore, the small number of studies thus far published have suggested that in humans, weight loss produced by CLA supplementation is at most modest.[10,13,16,18] To assess the efficacy of CLA as an aid to weight loss and its effect on cardiovascular risk factors, we undertook a double blind study examining the effects of a commercially available isomeric mixture of CLA on body weight, body fat mass and distribution, endothelial function, insulin sensitivity, and markers of oxidative stress in overweight middle-aged men.

Methods

Subjects

Forty nonsmoking white men, aged 35 to 60, without diabetes, hypertension, or cardiovascular disease, with a body mass index (BMI) >27 kg/m^2 were recruited from the local community through media advertisements. All subjects gave informed written consent and the protocol was approved by the local research and ethics committee.

Protocol

Subjects were randomly assigned to receive 4.5 g/d of CLA(isomeric mixture 60 calories/d) or olive oil(54 calories/d). The randomization was performed by an independent observer who also matched subjects by age and BMI. The isomeric mixture contained 35% 9c,11t CLA, 36% t10,c12 CLA, 1% to 2% 9c,11c and 10c,12c CLA, 1.5% 9t,11t and 10t,11t CLA, and <1% t8,c10 and c11,t13 CLA. The CLA and olive oil capsules were supplied by Natural Lipids (Hovdebygda, Norway). All vascular measurements were made in the morning after an overnight fast, at the beginning of the study, and after 12 weeks of supplementation.

Body Composition

Body weight and height were measured and BMI calculated. Skinfold thicknesses were measured according to standard guidelines[19] with Harpenden skinfold calipers (Holtain Ltd, Crymych, UK) at the following sites: biceps, triceps, front mid-thigh, medial calf, subscapular, mid-axillary, and abdominal. Abdominal, waist, and hip girths were measured. All measurements were made in triplicate and averaged. Bioelectrical impedance analysis was performed using the

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tetrapolar method and a Bodystat 1500 analyser (Bodystat Ltd, Isle of Man). Abdominal adipose tissue and liver fat were measured using two computed tomography (CT) images as described previously.[20] Abdominal adipose tissue is presented as a surface area at the level of the fourth lumbar vertebrae, and hepatic and splenic fat is presented as radiographic density in Hounsfield units (HU). Images were acquired using a Somatom Plus 4 scanner. CT slices were 10 mm in thickness and were obtained at 120 kV and 200 mA, with a 42-cm field of view and a 512×512 matrix. Image analysis was performed using dedicated software (SliceOmatic Version 4.2; Tomovision, Montreal, Canada).

Endothelial Function

Changes in brachial artery diameter in response to reactive hyperemia (FMD) were measured noninvasively using a high-resolution ultrasonic wall-tracking system (Vadirec Wall-track System™) as previously validated.[21,49,50] Studies were performed at a controlled temperature of 21°C, with subjects supine and their arm held outstretched on a cushion. Baseline measurements of internal brachial artery diameter were taken after 15 minutes of rest. Reactive hyperemia was produced by releasing a pediatric sphygmomanometer wrist cuff inflated to systolic pressure plus 50 mm Hg for 5 minutes. Internal brachial artery diameter was measured every minute after cuff release, and the maximum change from baseline was used to calculate FMD. Data are presented as the percentage diameter change from baseline in the brachial artery.

Laboratory Measurements

Venous blood samples were freshly analyzed for glucose, insulin, cholesterol (including low-density lipoprotein and high-density lipoprotein), and C-reactive protein. Further samples were centrifuged and the supernatant frozen at −80°C. These samples were analyzed later using enzyme immuno-linked assays for leptin (Alexis), adiponectin (Biogenesis UK), F2-isoprostanes (Alexis), and tumor necrosis factor-α (paired antibody enzyme-linked immunosorbent assay). Insulin sensitivity was measured indirectly using HOMA–IR [fasting serum insulin (μU/mL)×fasting plasma glucose (mmol/L)/22.5].

Statistical Analysis

Analysis was performed using SPSS v11.5. Baseline values are presented as mean±SD. Variables with a skewed distribution were logarithmically transformed to achieve a normal distribution prior to analysis. Baseline comparison between the CLA and olive oil groups was assessed by Student *t* test. The effects of treatment with CLA versus olive oil were assessed by using ANCOVA using baseline values as covariates and are presented with 95% confidence intervals. To avoid type I errors, post-hoc Bonferroni corrections were applied to the groups of primary objective measurements. Therefore, 2-tailed $P<0.025$, $P<0.005$, $P<0.001$, $P<0.025$, and $P<0.008$ were regarded as significant for blood pressure, skin-folds, girths, FMD, and abdominal CT measurements, respectively. A 2-tailed $P<0.05$ was regarded as significant for primary objective measurements weight, and bioimpedance, and the exploratory blood analyses.

Results

Baseline measurements did not differ significantly in the olive oil (19 subjects) and CLA (21 subjects) groups (Table 1). All subjects completed the study. The effect of 12 weeks of supplementation is shown in Table 2. The important changes are also displayed in the Figure. There were no significant changes in parameters in the olive oil group except for a decline in tumor necrosis factor-α (−61pg/mL [95% CI, −3 to −120]; $P=0.04$). In the CLA group there was no significant change in body mass (−1.1 kg [95% CI, −2.3 to 0.04]; $P=0.06$), BMI (−0.4kg/m² [95% CI, −0.8 to 0.03]; $P=0.07$), or total body fat (−1% [95% CI, −2.5 to 0.5]; $P=0.18$). There was a significant decrease in limb (−7.8 mm [95% CI, −11.1 to −4.5]; $P<0.001$), but not torso (−4.0 mm [95% CI, −11.5 to 3.5]; $P=0.29$) skin folds, and a significant increase in the total torso-to-limb skin-fold ratio(+0.13 [95%

TABLE 1. Characteristics of Olive Oil and CLA Groups at the Start of the Study

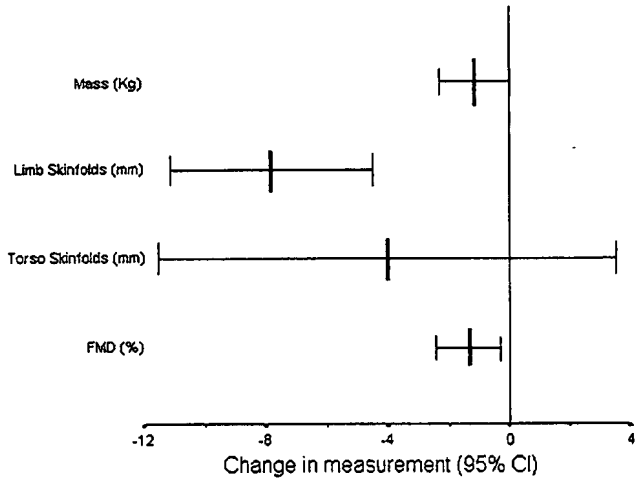
Baseline Characteristic	Olive Oil (n=19)	CLA (n=21)	*P*
Age, y	47±8	45±6	NS
Mass, kg	97±13	101±9	NS
BMI	33±3	33±3	NS
Fat, % (Bioimpedance)	29±3	28±4	NS
Systolic BP, mm Hg	128±13	122±10	NS
Diastolic BP, mm Hg	85±7	80±8	NS
Anthropometric measurements			
Skin-folds: Biceps, mm	12±4	11±4	NS
Skin-folds: Triceps, mm	20±5	19±5	NS
Skin-folds: Mid-thigh, mm	23±7	25±8	NS
Skin-folds: Medial calf, mm	16±4	16±5	NS
Skin-folds: Subscapular, mm	32±6	32±7	NS
Skin-folds: Mid-axillary, mm	23±4	24±6	NS
Skin-folds: Abdominal, mm	33±7	35±6	NS
Skin-folds: Limb, mm	72±17	72±18	NS
Skin-folds: Torso, mm	123±13	128±16	NS
Skin-folds: Torso:Limb	1.8±0.4	1.9±0.3	NS
Girth: Abdomen, cm	107±7	107±6	NS
Girth: Waist, cm	112±7	112±7	NS
Girth: Hip, cm	110±6	110±5	NS
Girth: Waist:Hip	1.02±0.03	1.01±0.04	NS
Girth: Abdominal:Hip	0.98±0.05	0.97±0.05	NS
Endothelial function			
Brachial artery, mm	3.8±0.5	3.7±0.4	NS
Brachial artery FMD, %	2.1±2.1	2.2±2.7	NS
Abdominal CT measurements			
Liver, HU	60±13	66±10	NS
Spleen, HU	64±2	61±2	NS
Liver:Spleen	0.93±0.18	1.08±0.18	NS
Visceral area, cm²	242±82	204±70	NS
Subcutaneous fat area, cm²	341±80	357±93	NS
Visceral:Subcutaneous fat	0.75±0.38	0.63±0.37	NS
Blood analyses			
LDL cholesterol, mmol/L	3.6±0.9	3.4±0.8	NS
HDL cholesterol, mmol/L	1.0±0.3	1.1±0.3	NS
Total cholesterol, mmol/L	5.6±0.9	5.4±0.8	NS
Triglycerides, mmol/L	2.3±1.3	1.8±1.0	NS
CRP, mg/L	5.2±2.9	4.8±2.7	NS
Glucose, mmol/L	5.2±0.6	5.1±0.7	NS
Insulin, mU/L	18±22	13±7	NS
HOMA-IR	4.6±6.4	3.1±1.8	NS
Leptin, ng/mL	21±12	22±16	NS
Adiponectin, pg/mL	8±6	9±6	NS
F2-isoprostanes, pg/mL	135±67	99±103	NS
Tumor necrosis factor-α, pg/mL	122±97	109±145	0.04

NS indicates not significant.

TABLE 2. Change in Measurements in Each Treatment Group After 12 Weeks of Supplementation Plus ANCOVA Analyses Showing the Overall Effect of Supplementation With CLA

	Olive Oil	CLA	Change In Measurement (ANCOVA) [95%CI]
Mass, kg	0.9±1.4	−0.2±1.9	−1.1 [−2.3 to 0.04] (*P*=0.06)
BMI	0.3±0.5	−0.1±0.7	−0.4 [−0.8 to 0.03] (*P*=0.07)
Fat, % (Bioimpedance)	1.1±2.9	0.1±1.7	−1 [−2.5 to 0.5] (*P*=0.18)
Systolic BP, mm Hg	0.2±6.7	−0.4±9.1	−2.2 [−7.1 to 2.7] (*P*=0.37)
Diastolic BP, mm Hg	−0.8±7.5	0.1±5.7	−1.3 [−5.4 to 2.8] (*P*=0.52)
Anthropometric measurements			
Skin-folds: Biceps, mm	0.1±2.3	−1.4±1.5	−1.6 [−2.7 to −0.5] (*P*=0.005)
Skin-folds: Triceps, mm	0.3±1.9	−1.1±2.4	−1.5 [−2.9 to 0.03] (*P*=0.046)
Skin-folds: Mid-thigh, mm	0.4±2.1	−2.6±2.6	−2.8 [−4.3 to −1.3] (*P*<0.001)
Skin-folds: Medial calf, mm	0.2±1.4	−1.5±1.5	−1.8 [−2.7 to −0.8] (*P*=0.001)
Skin-folds: Sub-scapular, mm	0.9±3.3	−0.3±4.8	−1.3 [−3.8 to 1.2] (*P*=0.29)
Skin-folds: Mid-axillary, mm	−0.4±2.8	−3.0±3.0	−2.3 [−4.0 to −0.6] (*P*=0.011)
Skin-folds: Abdominal, mm	2.0±6.8	2.2±3.4	+1.4 [−1.6 to 4.3] (*P*=0.36)
Skin-folds: Limb, mm	1.1±3.5	−6.6±6.0	−7.8 [−11.1 to −4.5] (*P*<0.001)
Skin-folds: Torso, mm	2.9±13.5	−2.1±9.9	−4.0 [−11.5 to 3.5] (*P*=0.29)
Skin-folds: Torso:Limb	0.02±0.18	0.15±0.15	+0.13 [0.03 to 0.24] (*P*=0.017)
Girth: Abdomen, cm	0.1±1.3	−0.1±1.9	−0.2 [−1.4 to 0.9] (*P*=0.69)
Girths: waist, cm	−0.3±2.1	0.5±2.5	+0.8 [−0.8 to 2.4] (*P*=0.30)
Girths: hip, cm	0.4±1.4	−0.01±1.5	−0.4 [−1.3 to 0.6] (*P*=0.44)
Girths: Waist:Hip	−0.003±0.03	0.01±0.22	+0.01 [−0.01 to 0.02] (*P*=0.44)
Girths: Abdominal:Hip	−0.01±0.03	−0.01±0.01	+0.01 [−0.01 to 0.02] (*P*=0.42)
Endothelial function			
Brachial artery, mm	−0.17±0.60	−0.03±0.62	+0.06 [−0.29 to 0.41] (*P*=0.72)
Brachial artery FMD, %	0.1±2.3	−1.3±2.5	−1.3 [−2.4 to −0.3] (*P*=0.013)
Abdominal CT measurements			
Liver, HU	−0.7±5.7	−4.1±10.2	−3.9 [−9.9 to 2.2] (*P*=0.20)
Spleen, HU	−2.2±4.8	0.7±2.8	1.1 [−1.7 to 3.8] (*P*=0.44)
Liver:Spleen	0.01±0.11	−0.08±0.17	−0.09 [−0.21 to 0.03] (*P*=0.12)
Visceral area, cm^2	11.1±34.5	3.7±55.0	−4.5 [−33.6 to 24.6] (*P*=0.76)
Subcutaneous fat area, cm^2	−9.8±32.6	3.43±40.0	−12.6 [−39.5 to 14.2] (*P*=0.34)
Visceral:Subcutaneous fat	−0.09±0.26	0.03±0.29	0.08 [−0.11 to 0.26] (*P*=0.41)
Blood analyses			
LDL cholesterol, mmol/L	−0.2±0.5	−0.01±0.4	+0.1 [−0.1 to 0.3] (*P*=0.41)
HDL cholesterol, mmol/L	−0.03±0.11	−0.09±0.13	−0.03 [−0.10 to 0.05] (*P*=0.46)
Total cholesterol, mmol/L	−0.09±0.50	−0.07±0.44	−0.07 [−0.34 to 0.20] (*P*=0.62)
Triglycerides, mmol/L	0.1±0.8	0.1±0.8	−0.1 [−0.6 to 0.4] (*P*=0.57)
CRP, mg/L	−0.8±2.9	0.4±4.8	+0.9 [−1.4 to 3.2] (*P*=0.41)
Glucose, mmol/L	0.3±0.8	0.2±0.8	−0.1 [−0.5 to 0.3] (*P*=0.51)
Insulin, mU/L	0.7±22.2	2.3±12.6	−3 [−11 to 6] (*P*=0.50)
HOMA-IR	0.2±6.7	0.8±3.2	−0.8 [−3.2 to 1.6] (*P*=0.50)
Leptin, ng/mL	1.3±7.3	0.7±5.1	−0.7 [−4.8 to 3.4] (*P*=0.72)
Adiponectin, pg/mL	0.5±3.4	−0.2±4.0	−0.2 [−2.4 to 2.0] (*P*=0.84)
F2-Isoprostanes, pg/mL	−36±95	94±200	+91 [3 to 178] (*P*=0.042)
Tumor necrosis factor-α, pg/mL	−61±70	15±21	+45 [−11 to 101] (*P*=0.11)

CI, 0.03 to 0.24]; $P=0.017$). There was no significant change in abdominal, waist, or hip girths, or in subcutaneous abdominal fat and liver fat measured by CT. However, there was a significant decrease in brachial artery FMD (−1.3% [95% CI, −2.4 to −0.3]; $P=0.013$), and a significant increase in plasma F2-isoprostanes(+91pg/mL [95% CI, 3 to 178]; $P=0.042$). There was no change in estimated insulin sensitivity, total cholesterol, low-density lipoprotein cholesterol,



Main findings of changes caused by CLA using baseline values as covariates (ANCOVA).

high-density lipoprotein cholesterol, triglycerides, CRP, leptin, or adiponectin.

There was a significant negative correlation between change in F2-isoprostanes and change in total limb skin folds (ie, loss of limb skin-fold thickness was associated with an increase in F2-isoprostanes) for the entire group (CLA+olive oil) ($P=0.012$) but no significant correlation when each group was analyzed separately. There was no significant correlation between change in endothelial function and change in F2-isoprostanes or change in limb skin-fold thicknesses.

Discussion

Obesity, and in particular abdominal obesity, is associated with increased cardiovascular risk,[22–24] and intentional weight reduction improves cardiovascular risk.[25,26] Recently there has been a great deal of interest in the effect of the Mediterranean diet on cardiovascular risk. A 2-year study examining the effect of the Mediterranean diet in patients with the metabolic syndrome found a reduction in body weight, an improvement in endothelial function, a decrease in CRP and an improvement in insulin resistance.[27] A study that tried to identify which component of the Mediterranean diet was responsible for improving cardiovascular risk paradoxically found that olive oil impaired endothelial function, although this was inversely correlated with changes in triglycerides.[28] The conclusion of the study was that it was the antioxidant and omega-3–rich foods that conferred cardiovascular benefit. Nevertheless, these studies supported the view that dietary modification or supplementation may have a significant impact on obesity and, in particular, cardiovascular risk.

Experimental evidence in animal models suggests that CLA supplementation, in particular the 10,12 CLA isomer, induces fat mass loss.[29–32] On the basis of this initial evidence, there has been a great deal of interest in its use as an aid to lose fat and weight in humans. Blankson et al reported that 12-week supplementation with >3.4 g/d isomeric CLA significantly reduced body fat mass in overweight volunteers, although there was no change in weight or BMI,[10] and Riserus et al found that only 4-week supplementation with 4.2 g/d isomeric CLA significantly improved sagittal abdominal diameter.[33] However, Zambell et al found no significant change in weight, BMI, or fat mass after 3-g/d supplementation with isomeric CLA (in women who were not overweight).[18] The observation in two studies of an impairment of insulin sensitivity have raised concerns.[13–15] This study was therefore designed to assess the effect of CLA supplementation on BMI, body fat distribution, and markers of cardiovascular risk, including endothelial function.

Our study found that an isomeric mixture of CLA did not cause significant weight loss (although there was a trend to weight loss of 1.1 kg). This is consistent with the 0.24 to 0.46 kg weight loss reported in previous studies.[13,18] Although CLA reduced limb fat, it had no effect on abdominal fat or liver fat(although there was a nonsignificant trend to an increase in liver fat, measured as a decrease in liver density in Hounsfield units). This finding is in contrast to a previous report that found a decrease in sagittal abdominal diameter after 4 weeks of 4.2 g/d CLA.[33] However, the suggestion that CLA may have a lipodystrophic effect is not new. A study supplementing mice with isomeric CLA found a reduction in fat mass, liver hypertrophy, and an increase in insulin resistance,[34] whereas mice fed the 10,12 CLA isomer had hyperinsulinemia and an increase in liver fat develop.[35] The mechanism for this is not clear, although a rapid decrease in leptin and adiponectin has been observed in mice only 2 days after starting CLA supplementation.[36] A decrease in leptin has also been observed in rats.[37] This hypothesis is supported by the observation that hyperinsulinemia and liver steatosis are partially reversed when hypoleptinemia is normalized by leptin infusion in CLA lipoatrophic mice.[34] However, there is conflicting evidence regarding the effects of CLA supplementation on plasma leptin in humans. One study supplementing patients with type 2 diabetes with CLA found a decrease in leptin,[38] but another study supplementing obese men with CLA found no change in leptin.[13] No clinical studies in humans have measured adiponectin after CLA supplementation. In contrast to these findings, a study supplementing Zucker diabetic fatty rats with CLA found that the previous impaired glucose tolerance improved.[39]

Cell culture and animal studies have suggested several other potential mechanisms by which CLA may reduce body fat, including reducing apolipoprotein B secretion in HepG2 cells,[40] increasing carnitine palmitoyltransferase activity and decreasing lipoprotein lipase activity,[30] and increasing tumor necrosis factor and uncoupling protein levels.[34] The role of peroxisome proliferator-activated receptor gamma (PPARγ) activity is not clear. PPARγ activity was increased in Zucker diabetic fatty rats and genetically obese mice fed CLA,[39,41,42] although several in vitro studies have found that 10,12 CLA downregulates PPARγ activity in mice adipocytes.[43,44] In vivo and in vitro studies in pigs have also found that CLA induced an increase in PPARγ activity.[45] However, human studies have found an opposite effect on PPARγ. Human adipocytes cultured in vitro with 10,12 CLA decreased the expression of PPARγ,[41,43] and diabetic patients treated with PPARγ agonists (glitazones) experience decreased central fat and increased peripheral fat.[46]

Our observations are important because reducing nonabdominal fat is less likely to reduce cardiovascular risk, and an increase in hepatic fat will increase insulin requirements.[47] Thus any weight loss with this regime is at most modest and the pattern of weight loss is not metabolically favorable.

Furthermore, we found that CLA significantly impaired brachial artery endothelial function, consistent with an adverse impact on cardiovascular risk.[51] The mechanism of this effect is not clear. We and others[16] found an increase in F_2 isoprostanes, a lipid peroxidation product generally considered to be a marker of increased oxidative stress. Another previous study reported an increase in plasma CRP, a marker of inflammation.[17] Taken together, these data suggest that this CLA regime impairs endothelial function and that this may, at least in part, be caused by increased oxidative stress. It is possible that the observed change in FMD in this study has been caused by the change in limb skin-fold thicknesses interfering with the FMD technique (for example, by altering wrist arterial occlusion pressure or changing depth from probe to brachial artery), thus giving a false measurement. However, we feel that this is unlikely because a high wrist arterial occlusion pressure was used and a change in depth to the brachial artery of 1 to 2 mm is well within the capability of the ultrasound probe. Brachial artery FMD measurements vary markedly from laboratory to laboratory, dependant on the exact technique used.[48] In this study, we used the wrist cuff technique, which results in lower values of brachial artery FMD than those obtained using the upper arm or mid-forearm techniques. We did not observe an adverse impact on insulin sensitivity. However, the study may have been under-powered to detect differences using the HOMA technique.[52] We did not identify any change in plasma lipid profiles.

The effect of CLA on other cardiovascular risk factors has been examined. One study reported that CLA decreased platelet aggregability,[53] but another reported no change in platelet aggregability.[54] A small decrease in total, low-density lipoprotein, and high-density lipoprotein cholesterol was found in overweight men taking 1.7 g/d CLA and 3.4 g/d CLA, although this was not maintained at higher doses.[10] A further study in obese men using 10,12 CLA and a CLA mixture lowered high-density lipoprotein cholesterol, although no change was observed in total or low-density lipoprotein cholesterol or triglycerides.[13] No change in lipids was found in a study with healthy women supplemented with 3.9 g/d CLA.[55]

Consistent with our observations, 4.2 g/d CLA for 1 month was found to increase urinary isoprostanes in men with abdominal obesity.[16] Isoprostanes are produced from peroxidation of lipids, and it was suggested that the increase in isoprostanes might be simply a result of increased fat lipolysis, rather than indicating increased oxidative stress. However, more recent studies have found that 10,12 CLA increases insulin resistance[13] and plasma CRP.[17] Taken together with our observation of an impairment of endothelial function, it seems highly likely that the increase in isoprostanes does indeed imply an increase in oxidative stress.

Conclusions

CLA supplementation for 12 weeks using the regime used in this study had no significant effect on BMI. Even if this represents a type 2 error, the reduction is at most modest, consistent with previous reports in man. Furthermore, the pattern of fat loss is peripheral rather than central. Importantly, the observed impairment of endothelial function and increase in markers of oxidative stress raise concerns about the widespread use of this agent until further studies demonstrate its cardiovascular safety or otherwise.

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ADVERSE CARDIOVASCULAR AND CENTRAL NERVOUS SYSTEM EVENTS ASSOCIATED WITH DIETARY SUPPLEMENTS CONTAINING EPHEDRA ALKALOIDS

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ABSTRACT

Background Dietary supplements that contain ephedra alkaloids (sometimes called ma huang) are widely promoted and used in the United States as a means of losing weight and increasing energy. In the light of recently reported adverse events related to use of these products, the Food and Drug Administration (FDA) has proposed limits on the dose and duration of use of such supplements. The FDA requested an independent review of reports of adverse events related to the use of supplements that contained ephedra alkaloids to assess causation and to estimate the level of risk the use of these supplements poses to consumers.

Methods We reviewed 140 reports of adverse events related to the use of dietary supplements containing ephedra alkaloids that were submitted to the FDA between June 1, 1997, and March 31, 1999. A standardized rating system for assessing causation was applied to each adverse event.

Results Thirty-one percent of cases were considered to be definitely or probably related to the use of supplements containing ephedra alkaloids, and 31 percent were deemed to be possibly related. Among the adverse events that were deemed definitely, probably, or possibly related to the use of supplements containing ephedra alkaloids, 47 percent involved cardiovascular symptoms and 18 percent involved the central nervous system. Hypertension was the single most frequent adverse effect (17 reports), followed by palpitations, tachycardia, or both (13); stroke (10); and seizures (7). Ten events resulted in death, and 13 events produced permanent disability, representing 26 percent of the definite, probable, and possible cases.

Conclusions The use of dietary supplements that contain ephedra alkaloids may pose a health risk to some persons. These findings indicate the need for a better understanding of individual susceptibility to the adverse effects of such dietary supplements. (N Engl J Med 2000;343:1833-8.)

DIETARY supplements that contain ephedra alkaloids (also known as ma huang) and guarana-derived caffeine are widely consumed in the United States for purposes of weight reduction and energy enhancement. A number of reports of adverse reactions to dietary supplements that contain ephedra alkaloids, some of which resulted in permanent injury or death, have appeared in the medical literature.[1-6] In response to growing concern about the safety of ephedra alkaloids in dietary supplements, the Food and Drug Administration (FDA) requested an independent review of reports of adverse events related to the use of ephedra alkaloids to assess causation and determine the level of risk these products pose to consumers.

We conducted an in-depth review of 140 reports of adverse events involving dietary supplements containing ephedra alkaloids that were submitted to the FDA between June 1, 1997, and March 31, 1999, and applied a standardized rating system for assessing causation. We also evaluated factors that might increase the risk to consumers and the adequacy of warnings about potential risks included on product labels. The full report of our review of adverse events is available elsewhere.[7] Here, we summarize our findings.

METHODS

The objective of the review was to determine the likelihood that ephedra alkaloids (which were usually combined with caffeine) caused the reported adverse events on the basis of the information provided in the FDA MedWatch report, along with supplemental medical records. We independently reviewed each of the 140 cases. Causation was assessed according to the criteria described by Blanc et al.[8] and included an evaluation of the timing of the event in relation to the dose and duration of use of a product; an assessment of the pattern of response to determine whether it constituted a recognized reaction to the substance on the basis of previous reports of ephedrine or similar stimulants in the medical literature; and a determination of the contribution of any underlying diseases or medical conditions.

In general, we defined an adverse event as definitely related to the use of supplements containing ephedra alkaloids only if the symptoms recurred with the reintroduction of ephedra alkaloids or when the onset of symptoms coincided with the expected peak plasma concentration of the drug and resolved within an interval that was consistent with the expected duration of the effect of ephedrine. An adverse event was defined as probably related to the use of supplements containing ephedra alkaloids when the majority of the evidence supported the existence of a causal link but one or more aspects of the case, such as time since the last dose, were unknown or there was a minor inconsistency in the supporting evidence, such as a low reported dose. An adverse event was designated as possibly related to the use of supplements containing ephedra alkaloids when it was equally likely that the adverse event was not related to the use of ephedra alkaloids; for example, in the case of effects that have not been reported in the literature in association with ephedra alkaloids but that are pharmacologically plausible. Reports of ad-

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verse events that included scant medical history and incomplete information about the product involved were usually considered to have insufficient information to be assessed. This category was reserved for events in which the evidence was not substantial enough to consider them as possibly related to the use of supplements containing ephedra alkaloids. Adverse events were defined as probably unrelated to the use of supplements containing ephedra alkaloids if the evidence that ephedra alkaloids were the cause was weak or if the likelihood was strong that there was some other cause, either medical or toxicologic. When the scientific evidence or course of events was highly inconsistent with the known effects of ephedra alkaloids, the event was considered definitely unrelated; for example, in the case of symptoms that persisted long after the use of ephedra alkaloids had been discontinued or in the case of symptoms that had no association with the known pharmacodynamic effects of ephedra alkaloids. However, the event was considered related if the patient had a preexisting condition such as hypertension that could have been aggravated by the use of ephedra alkaloids and if the pattern of use met the criteria for causation.

In determining the likelihood of a causal link, we evaluated aspects of the medical history, dietary patterns, and social habits as possible contributing or causative factors. For example, we noted when events occurred while patients were fasting or in conjunction with high intakes of caffeine. We recognized that in the case of adverse events that were most likely not related to the use of supplements containing ephedra alkaloids one or more of the other ingredients in the supplement may have been causally related to the event.

RESULTS

Features of the Cases

The age and sex of the users of products containing ephedra aklaloids and the reported reasons for use are shown in Table 1. Although the labels of most such products state that they are not intended for use by persons less than 18 years of age, adverse events were recorded in at least 10 persons under this age. The youngest was 15 years old. Overall, 43 cases (31 percent) were considered to be definitely or probably related to the use of supplements containing ephedra alkaloids, 24 cases (17 percent) were considered to be unrelated to the use of such supplements, 44 cases (31 percent) were deemed possibly related to the use of such supplements, and in 29 cases (21 percent) the information provided was insufficient to assess causation. The types of adverse events that were definitely or probably related to the use of supplements containing ephedra alkaloids and those that were possibly related are summarized in Table 2.

Cardiovascular symptoms made up 47 percent of the adverse events that were definitely, probably, or possibly related to the use of supplements containing ephedra alkaloids. Hypertension was the single most frequent adverse effect, followed by palpitations, tachycardia, or both. Eighteen percent of related and possibly related adverse events involved the central nervous system. Strokes (n=10) and seizures (n=7) were the most frequent type of central nervous system event reported. The clinical outcomes of the definite, probable, and possible cases are listed in Table 3.

Ten events resulted in death (including 1 neonatal death and 1 fetal death), and 13 events resulted in permanent impairment, which represented 26 percent of the definite, probable, and possible cases. Nine serious adverse events occurred in persons who were taking relatively low doses of ephedra alkaloids (range, 12 to 36 mg per day) and who had no important medical risk factors. Features of the definite or probable and possible cases that resulted in death or permanent impairment or that necessitated substantial medical intervention are given in Tables 4 and 5, respectively.

Of the sudden catastrophic cerebrovascular and cardiovascular events, 11 occurred in previously healthy persons. Some of these cases, which were definitely or probably related to the use of supplements containing ephedra alkaloids, are described in detail in the following sections.

TABLE 1. AGE AND SEX OF USERS OF SUPPLEMENTS CONTAINING EPHEDRA ALKALOIDS AND REASONS FOR USE, ACCORDING TO ADVERSE-EVENT REPORTS.

VARIABLE	NO. OF USERS (%)
Age	
<18 yr	10 (7)
18–29 yr	34 (24)
30–45 yr	45 (32)
>45 yr	32 (23)
Unknown	19 (14)
Sex	
Male	56 (40)
Female	84 (60)
Reason for use	
Weight loss	83 (59)
To increase athletic performance	23 (16)
To increase energy	9 (6)
Unknown	24 (17)
Intentional misuse	1 (1)

Examples of Severe Cerebrovascular Adverse Events

Patient 1

Patient 1 was a healthy 35-year-old woman who had taken aerobic-exercise classes for several years without incident (Table 4). In July 1997, she began taking one capsule of Shape-Fast Plus (according to the label, each capsule contained 15 mg of ephedra alkaloids and 40 mg of caffeine) three times a day before meals for weight loss; she was taking no other medications. She had been taking the product for one week when she collapsed during an aerobics class. Bystanders observed that her arms and legs were flexing and tensing. In the emergency department, her blood pressure was 110/38 mm Hg and the heart rate was 104 beats per minute. A computed tomographic scan of the head showed a subarachnoid hemorrhage. Cerebral angiography showed no evidence of a vascular aneurysm. A urine toxicology screen was positive for amphetamine, a result presumed to reflect a cross-reaction with the ephedrine and therefore to be false positive.

TABLE 2. TYPES OF ADVERSE EVENTS THAT WERE DEFINITELY, PROBABLY, OR POSSIBLY RELATED TO THE USE OF SUPPLEMENTS CONTAINING EPHEDRA ALKALOIDS.

ADVERSE EVENT	DEFINITELY OR PROBABLY RELATED (N=43)	POSSIBLY RELATED (N=44)	TOTAL (N=87)
	no. of events (%)		
Cardiovascular			
Hypertension	10 (21)	7 (14)	17 (17)
Palpitations, tachycardia, or both	8 (17)	5 (10)	13 (13)
Arrhythmia	3 (6)	3 (6)	6 (6)
Myocardial infarction	2 (4)	0	2 (2)
Cardiac arrest or sudden death	5 (10)	3 (6)	8 (8)
Central nervous system			
Stroke	4 (8)	6 (12)	10 (10)
Transient ischemic attack	1 (2)	0	1 (1)
Seizure	1 (2)	6 (12)	7 (7)
Other	14 (29)	20 (40)	34 (35)
Total no. of events*	48	50	98

*The total number of events exceeds the total number of cases, since some cases involved more than one adverse event.

TABLE 3. CLINICAL OUTCOMES OF ADVERSE EVENTS THAT WERE DEFINITELY, PROBABLY, OR POSSIBLY RELATED TO THE USE OF SUPPLEMENTS CONTAINING EPHEDRA ALKALOIDS.

OUTCOME	DEFINITELY OR PROBABLY RELATED (N=43)	POSSIBLY RELATED (N=44)	TOTAL (N=87)
	no. of events (%)		
Death	3 (7)	7 (16)	10 (11)
Permanent impairment	7 (16)	6 (14)	13 (15)
Ongoing medical treatment	4 (9)	4 (9)	8 (9)
Full recovery	29 (67)	13 (30)	42 (48)
Unknown	0	14 (32)	14 (16)

Neurogenic pulmonary edema rapidly developed, necessitating endotracheal intubation and mechanical ventilation. Electrocardiographic findings and cardiac-enzyme levels were consistent with the occurrence of a small myocardial infarction. The treating cardiologist and neurologist thought that ephedrine induced the subarachnoid hemorrhage. The finding of amphetamine on the urine toxicology test supports the presence of ephedrine at the time of the event. Laboratory analysis of the supplement determined that the ephedrine content was 12.0 mg per capsule. At that time, the FDA's recommendation was a maximal dose of 8 mg per serving.[7]

Patient 10

Patient 10 was an apparently healthy 39-year-old man who experienced numbness of the right arm and leg on March 17, 1998, 90 minutes after drinking Ultimate Orange, which according to the label contained 415 mg of ma huang (ephedra alkaloids) per serving as well as guarana (a source of caffeine), and 5 minutes after running 3 miles (4.8 km) (Table 4). He also regularly took multivitamins and amino acid supplements, but no other medications. On presentation at a nearby hospital, his blood pressure was 140/78 mm Hg and his pulse was 60 beats per minute. A computed tomographic scan of the head revealed a left-sided intrathalamic hemorrhage. Cerebral angiography showed no evidence of vascular anomalies. The patient had gradual clinical improvement, and his symptoms resolved except for persistent sensory loss on the right side of his face. Chemical analysis of the Ultimate Orange product confirmed the presence of ephedrine, as well as of pseudoephedrine, norephedrine, and norpseudoephedrine.

Examples of Severe Cardiovascular Adverse Events

Patient 2

Patient 2 was a 22-year-old man with a history of asthma who collapsed while lifting weights at a gym on March 31, 1998 (Table 4). His medications included theophylline (Theo-Dur; 300 mg twice daily), albuterol (Ventolin; administered as necessary through a metered-dose inhaler), and a combination of chlorpheniramine maleate, phenylephrine hydrochloride, and phenylpropanolamine hydrochloride (Atrohist Plus SR). According to friends, he had consumed one 18-oz bottle of Ripped Force (which is listed as containing 20 mg of ephedrine alkaloids, 100 mg of caffeine, 250 mg of L-carnitine, and 240 μg of chromium) before working out and was regularly drinking three bottles of Ripped Force per day. He also took creatine and protein supplements. Witnesses reported that he had a seizure. Paramedics initially found him apneic and in ventricular fibrillation. He was successfully resuscitated. Computed tomography of the head showed cerebral edema but no hemorrhage or masses. An initial electrocardiogram showed atrial flutter, which subsequently converted to sinus rhythm. An echocardiogram revealed mild left ventricular hypertrophy. The plasma theophylline level was 11 μg per milliliter (therapeutic range, 10 to 20), and urinalysis revealed 12 μg of ephedrine per milliliter, 0.38 μg of pseudoephedrine per milliliter, and 0.41 μg of phenylpropanolamine per milliliter. The treating cardiologist thought that the combination of ephedra alkaloids and caffeine in Ripped Force and the theophylline and albuterol medications caused a ventric-

TABLE 4. OUTCOME IN 11 PATIENTS WITH ADVERSE EVENTS THAT WERE DEFINITELY OR PROBABLY RELATED TO THE USE OF SUPPLEMENTS CONTAINING EPHEDRA ALKALOIDS.

PATIENT NO.	AGE (YR)/ SEX	NAME OF SUPPLEMENT	ESTIMATED DAILY DOSE OF EPHEDRA ALKALOIDS	DURATION OF USE	ADVERSE EVENT	OUTCOME	PREEXISTING CONDITIONS OR CONCURRENT RISKS
			mg				
1	35/F	Shape-Fast Plus	45	1 wk	Subarachnoid hemorrhage	Permanent disability	None
2	22/M	Ripped Force	20–60	Unknown	Arrhythmia, cardiac arrest	Permanent disability	Asthma
3	28/F	Herbalife's Thermo-jetics	21	1 day	Cardiac arrest	Permanent disability	None
4	43/M	Ripped Fuel	60	7 mo	Cardiac arrest	Death	Family history of coronary artery disease
5	37/F	Metabolife 356	36	1 wk	Severe hypertension, cardiac arrest, hypokalemia	Death	None
6	59/F	OmniTrim Extra Vitamin-Fortified tea	36	3 wk	Acute myocardial infarction	Coronary bypass surgery	Hypertension
7	38/M	Ripped Fuel	20	1 yr	Arrhythmia, cardiac arrest	Death	None
8	47/F	Total Control	44–66	9 mo	Hypertension, bilateral lacunar infarctions	Permanent disability	Concomitant ingestion of caffeine and ethanol
9	29/M	Ultimate Orange	30	2 wk	Stroke	Permanent disability	Concomitant use of dehydroepiandrosterone and androstenedione
10	39/M	Ultimate Orange	Unknown	Unknown	Hemorrhagic stroke	Permanent disability	None
11	47/M	Purple Blast	Unknown	3 wk	Hemorrhagic stroke	Permanent disability	Possible hypertension

ular arrhythmia that resulted in cardiac arrest. The patient suffered anoxic encephalopathy and remained in a vegetative state for several weeks. After one month in an acute care facility and six weeks at a rehabilitation facility, he was discharged with substantial residual neurologic impairment.

Patient 7

Patient 7 was an apparently healthy 38-year-old man who had been taking two capsules of Ripped Fuel (according to the label each capsule contains 10 mg of ephedrine and 100 mg of caffeine) each morning for one year as directed on the product label (Table 4). On June 6, 1996, he took his usual dose along with a cup of coffee and went jogging for 20 minutes. After returning home, he was talking with his family when he suddenly collapsed and appeared to have a tonic–clonic seizure. He had not reported any symptoms before collapsing. He was in full cardiac arrest when paramedics arrived and could not be resuscitated. Autopsy showed mild cardiomegaly with four-chamber dilatation and coronary artery disease, with narrowing of 50 to 75 percent in four vessels. The cause of death was acute arrhythmia resulting from atherosclerotic cardiovascular disease. Subsequent toxicology testing showed blood levels of 110 ng of ephedrine per milliliter (the therapeutic range used for bronchodilation is 20 to 80).[9] An addendum to the autopsy report included the comment, "ephedrine is a stimulant medication, and as such may have contributed to a fatal arrhythmia in the decedent."

DISCUSSION

Ephedrine and related alkaloids have been associated with adverse cardiovascular events, including acute myocardial infarction, severe hypertension, myocarditis, and lethal cardiac arrhythmias.[10,11] Constriction of coronary arteries and, in some cases, vasospasm are believed to be the mechanisms of myocarditis and myocardial infarction. The adrenergic effects of ephedrine shorten cardiac refractory periods, permitting the development of reentrant cardiac arrhythmias. Ephedrine can predispose patients to both hemorrhagic and ischemic stroke.[12] Subarachnoid hemorrhage is thought to be a result of the hypertensive action of ephedrine, which can be short lived, or of cerebral vasculitis, which has been described in association with a variety of sympathomimetic drugs.[13,14] Thrombotic stroke is presumably related to vasoconstriction of large cerebral arteries, which leads to local thrombosis as a result of stasis and sympathomimetic-induced platelet activation.

Caffeine is present in many products that contain ephedra alkaloids, and those who take these products might also be consuming considerable quantities of caffeine in coffee, tea, and soft drinks. Caffeine is like-

TABLE 5. OUTCOME IN 15 PATIENTS WITH ADVERSE EVENTS THAT WERE POSSIBLY RELATED TO THE USE OF SUPPLEMENTS CONTAINING EPHEDRA ALKALOIDS.

PATIENT NO.	AGE (YR)/ SEX	NAME OF SUPPLEMENT	ESTIMATED DAILY DOSE OF EPHEDRA ALKALOIDS	DURATION OF USE	ADVERSE EVENT	OUTCOME	PREEXISTING CONDITIONS OR CONCURRENT RISKS
			mg				
1	46/M	Diet Fuel	Unknown	5–6 mo	Stroke	Death	None
2	22/M	Ripped Fuel	Unknown	Unknown	Hyperthermia, abnormal electrolyte levels, cardiac arrest	Death	None
3	64/F	Fit America Natural Weight Control Aid	Unknown	2 mo	Atrial fibrillation, stroke	Permanent disability	Hypertension, transient ischemic attack
4	47/F	Per-Form Dieter's Natural Tea	Unknown	6 mo	Rhabdomyolysis, hydronephrosis, hypokalemia	Prolonged hospital care	None
5	64/F	Shape-Fast	20	Unknown	Hemorrhagic stroke	Permanent disability	None
6	34/M	Herbalife's Thermojetics	Unknown	>3 wk	Atrial flutter, renal failure, hypokalemia, rhabdomyolysis	Death	None
7	32/F	Ripped Fuel	20	4 yr	Premature delivery (34 wk of gestation)	Death of neonate	Smoking
8	29/M	Ultimate Nutrition Product Ma Huang	Unknown	6–7 mo	Stroke	Permanent disability	None
9	15/F	Ripped Fuel	Unknown	2–3 wk	Arrhythmia, cardiac arrest	Death	None
10	41/F	Diet-Phen	12	1 mo	Hypertension, multiple brainstem infarcts	Permanent disability	Possible hypertension
11	22/F	Magic Herb	72	3 mo	Spontaneous abortion at 9 wk	Death of fetus	None
12	43/F	Metabolife 356	12	6 mo	Severe hypertension, hemorrhagic stroke	Permanent disability	None
13	18/M	Ultimate Orange	Unknown	Unknown	Seizure, hemorrhagic stroke	Death	None
14	61/F	Metabolife 356	24; increased to 60	1 mo	Hypertension, unstable angina	Coronary bypass surgery	Asthma
15	26/M	Ripped Fuel	20–60	3 yr	Status epilepticus, hypokalemia	Permanent disability	None

ly to enhance the cardiovascular and central nervous system effects of ephedrine. Caffeine acts by competitively antagonizing the receptors for adenosine, a hormone released by endothelial cells that dilates blood vessels.[15] By inhibiting adenosine-mediated dilatation of blood vessels, caffeine constricts blood vessels and may increase blood pressure in persons prone to hypertension. Caffeine also augments the release of catecholamines, an effect that, when combined with that of ephedrine, could lead to increased stimulation of the central nervous system and cardiovascular system.[16]

Phenylpropanolamine, another ephedrine alkaloid, was marketed with caffeine in various weight-reducing aids until 1983, when the combination was banned by the FDA after numerous reports of adverse effects. Several studies have shown that caffeine and phenylpropanolamine have an additive effect on blood pressure.[17] These interactions between phenylpropanolamine and caffeine support the idea that the combination of ephedrine and caffeine in a dietary supplement could increase the risk of adverse effects.

The quantity of ephedrine in dietary supplements, as reported on package labels, is typically about 20 mg per serving, and the usual dose frequency is two to three times per day. These products may contain larger or smaller amounts of ephedra alkaloids than are listed on the product label. For example, 11 of 20 supplements tested by Gurley et al.[18] either failed to list the alkaloid content on the label or had more than a 20 percent difference between the amount listed on the label and the actual amount.

Often, the dose of ephedrine that was associated with an adverse event was less than a typical dose of ephedrine used for bronchodilation (25 to 50 mg). Experimental studies show that ephedrine has only moderate effects on heart rate and blood pressure at these doses.[19,20] The discrepancy between such data and our findings of serious adverse events reported with the use of dietary supplements containing ephedra alkaloids may be due to individual susceptibility, the additive stimulant effects of caffeine, the variability in the contents of pharmacologically active chemicals in the products, or preexisting medical conditions.

Many of the cases we reviewed involved side effects

such as anxiety, tremulousness, insomnia, palpitations, and personality changes that are well known to occur with the use of stimulant drugs. When ephedrine is used for medical purposes, these types of reactions are considered side effects and must be included in the assessment of risks and benefits. In fact, ephedrine is rarely prescribed today for medical purposes, because newer drugs have more specific actions and fewer side effects. The risks of taking ephedra alkaloids as a dietary supplement, however, are difficult to justify because the alkaloids have no demonstrated benefit. Unlike vitamins and minerals, ephedra alkaloid supplements are not essential for proper nutrition. People who take these products to increase their exercise capacity or to lose weight place themselves at risk without a substantial likelihood of benefit.

A limitation to the use of reports of adverse events as an indicator of a product's safety is that the number of people at risk for the event is unknown. Manufacturers of dietary supplements that contain ephedra alkaloids reported that 3 billion servings were sold in 1999.[21] The number of servings that were actually consumed is difficult to determine. Assuming that the products were consumed as directed — three doses per day for 12 weeks — then approximately 12 million people used these supplements in 1999.

Another limitation is that adverse events are known to be underreported. Several studies have shown that spontaneous reporting of adverse events to MedWatch is not routine, and the rate of reporting may be less than 15 percent.[22,23] The frequency of reports of adverse reactions to herbal products is thought to be even lower.[7,24] Therefore, the frequency of serious adverse events associated with the use of supplements containing ephedra alkaloids cannot be precisely determined with the use of current reporting mechanisms.

Because of the severity of the adverse events that we reviewed and, in particular, the occurrence of events that caused permanent disability and death, we conclude that dietary supplements that contain ephedra alkaloids pose a serious health risk to some users. Although the incidence of serious adverse effects cannot be determined from our analysis, our findings arouse concern about the risks of these products, given that they have no scientifically established benefits. Our findings indicate the need for a better understanding of the determinants of individual susceptibility to the serious adverse effects of dietary supplements containing ephedra alkaloids so that appropriate dosing guidelines and warnings can be devised.

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